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SAFE HANDLING OF INSECT CHEMOSTERILANTS IN RESEARCH AND FIELD USE

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SAFE HANDLING OF INSECT CHEMOSTERILANTS

IN RESEARCH AND FIELD USE

By Alexej B. Borkovec[1/]

Biological specificity of chemical compounds is seldom absolute. The ideal pesticide, ie., a chemical harmless to all organisms except one particular species of pests, is the ultimate goal but probably will never be reached. Since toxicology is essentially concerned only with the quantitative relations between the agent and the biological subject, the problem of safety in handling chemical compounds requires the understanding of the potential and the practical possibility of contaminating nontarget organisms by harmful amounts of the chemical.

When chemosterilants are used in the laboratory or in the field, the person who handles them should not only follow the general precautions pertaining to potentially toxic chemicals but he should also be familiar with the special characteristics of the particular chemosterilant. This is not an easy matter, considering the wide variety of chemicals with demonstrated sterilizing activity, and those workers who conduct exploratory research with new compounds must handle them with utmost care. However, only a few chemosterilants are being used extensively in laboratories and in field experiments. In this report the characteristics of these better known chemosterilants are described and procdures are suggested for handling them with relative safety.

CLASSIFICATION AND PROPERTIES OF CHEMOSTERILANTS

Because the development of chemosterilants is still in progress, no definitive statements can be made about all types of compounds with sterilizing activity. Over 1,000 such compounds have been described in the literature (4),[2/] but fortunately the few chemosterilants that are commonly used belong to chemically well-defined classes.

Alkylating Agents.--This is by far the most numerous and most broadly active class of chemosterilants (5). As the name implies, alkylating agents are moderately to highly reactive compounds capable of introducing a positively charged or an electrophilic moiety into a suitable nucleophilic receptor. The electrophile is usually a substituted alkyl group, but the receptor molecule may be extremely variable.

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2/ Underscored numbers in parentheses refer to Literature Cited at the end of this report.

In an organism, nucleic acids and proteins are usually considered the most probable targets; however, from a practical standpoint, hydrolysis and polymerization may be equally frequent reactions of alkylating agents. In hydrolysis the receptor is water and the interaction leads invariably to a loss of sterilizing activity of the agent. In polymerization the receptor is another molecule of the alkylating agent, and the result is loss of activity usually accompanied with a decrease in solubility. For toxicology, the first class of reactions is important, and because all cells contain potential receptors, the activity of alkylating agents is not restricted to pests but includes all living organisms. The second class of reactions, hydrolysis and polymerization, must be considered in handling, storing, and applying the chemosterilant and in maintaining its efficacy. Nevertheless the same reactions, because they lead to inactivation and detoxification, may influence the safety of the compound and its effects on environmental contamination.

If unwanted chemosterilants need to be disposed of or destroyed, their aqueous solutions can be mixed with a 5 percent solution of sulfuric or hydrochloric acid. The degradation is complete in minutes and the resulting products will be usually much less toxic than the original chemosterilants. Alkylating chemosterilants are not particularly sensitive to light and to oxidation by air, but high humidity and carbon dioxide may hasten their degradation. This consideration is important when the use of such chemosterilants for coatings or similar applications is contemplated.

The two most widely investigated types of alkylating chemosterilants are aziridines and alkanesulfonates. Chemical and physical properties of these compounds are extremely variable, but in a qualitative way they are closely related toxicologically--all are cytotoxic and mutagenic (15). Carcinogenic and teratogenic properties have not been clearly demonstrated for aziridinyl chemosterilants, but this type of toxicity must always be considered as possible or even probable. It is clear, therefore, that the potential hazards of these compounds are twofold--the acute intoxication by ingestion, inhalation, or contact and a more insidious chronic intoxication resulting from long-term exposures. Although the acute oral or intraperitoneal (ip.) LD_{50} in rats is but a very crude and imperfect indicator of the toxicity of a compound, it nevertheless provides the person who handles chemosterilants with an approximate gage for regulating his behavior and cautiousness. The LD_{50} of several aziridinyl and alkanesulfonate chemosterilants is shown in table 1.

Except for tretamine, which is highly toxic and reactive and which should not be used in any but purely basic research work, the acute toxicity of alkylating agents is not excessive, and the compounds can be handled safely when the basic precautions pertaining to handling any toxic compounds are followed. A more serious problem is the application of alkylating chemosterilants in the field. Although their reactivity is favorable for rapid degradation, the possible contamination of large areas, even with small residues of alkylating agents, makes their use as crop sprays or dusts hazardous and generally undesirable. On the other hand, a safe application is possible when these chemosterilants are used to sterilize reared or collected insects under controlled laboratory conditions and when the personnel is adequately protected.

Table 1.--Summary of physical and toxicological characteristics of various chemosterilants

Chemosterilant	Vapor pressure[1]	Solubility[2]	Acute oral LD_{50} in rats	References[3] to Toxicology	Analytical methods	Metabolism
	μ Hg.		Mg. per kg.			
Apholate; 2,2,4,4,6,6-hexakis=(1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-tri=azatriphosphorine; ENT-26316	---	Water, methanol, acetone.	98	15	2,6,7,8	---
Tepa; tris(1-aziridinyl)phos=phine oxide; ENT 24915	2.2	---do---	37	15,16,32	2,6,7,8,9 13,24,29	10,14,24, 26
Metepa; tris(2-methyl-1-aziridinyl)phosphine oxide; ENT-50003	5.5	---do---	136	15,16,23	2,6,7,8	28
Thiotepa; tris(1-aziridinyl)=phosphine sulfide; ENT-24916	1.2	---do---	[4]9	3,16	24,29	3,24,27
Tretamine; 2,4,5-tris(1-aziridinyl)s-triazine; ENT-25296	---	---do---	1	15,16,32	6	26
TMAC; 1,4-tetramethylenebis=(1-aziridinecarboxamide); ENT-50838	---	---do---	---	----	33	35
HMAC; 1,6-hexamethylenebis=(1-aziridinecarboxamide); ENT-50172	<.1	---do---	---	----	33	----

Table 1.--Continued

Busulfan; 1,4-butanediol dimethanesulfonate; ENT-25012	<1	Acetone, 95-percent ethanol.	5/ 18	25	31,34	26,36
Hempa; hexamethylphosphoric triamide; ENT-50882	102	Water, methanol, acetone.	2,650 ♂; 3,360 ♀	16,17,20, 30	1,6,12	12,18,19
Thiohempa; hexamethylphosphoro= thioic triamide; ENT-50918	20	Methanol, acetone.	---	----	----	18,19
Hemel; hexamethylmelamine; ENT-50852	---	Dilute acids, acetone.	350	16,17,22	11	11

1/ Measured at 27°-28° C.

2/ In secess of 3 g. per 100 ml.

3/ See literature cited at end of this report.

4/ Administered intravenously.

5/ Administered intraperitoneally.

Phosphorus Amides.--Although some of the most effective aziridinyl chemosterilants, e.g., tepa and thiotepa, are amides of phosphoric or phosphorothioic acid, they are classified as alkylating agents because the high reactivity of the aziridine ring determines their chemical and biological behavior. The phosphorus amides, on the other hand, do not contain alkylating functional groups and they do not react directly with nucleophilic receptors. Only two compounds in this category have received wide attention--hempa (table 1), a fully methylated amide of phosphoric acid, and thiohempa (table 1), an analogous derivative of phosphorothioic acid. Both compounds have similar physical, chemical, and biological properties and their handling and safety aspects are similar. The toxicology of thiohempa is not known, but hempa has been investigated in some detail. Although the acute toxicity of hempa (table 1) is very low, the compound is nevertheless cytotoxic and mutagenic but in much higher doses than tepa or most other alkylating agents. In rats, hempa exhibited no teratogenic or carcinogenic activity. The phosphorus amides are generally unreactive, thermally stable, and they are not readily attacked by dilute acids or bases. Their residues in nature would probably be more durable than those of the alkylating agents, but hempa and thiohempa are rapidly degraded by microsomal enzymes and their persistence in living organisms is low.

Triazines.--At present only hemel (table 1) and some of its lower methylated homologs are of interest. Most triazinyl chemosterilants are thermally stable and unreactive solids; some are sufficiently basic to form water-soluble salts with inorganic acids, and solutions of these compounds are strongly acidic and corrosive. Because of the close similarity between sterilizing and herbicidal trizaines, the former should be used with caution when plants or their root systems could be contaminated. All triazines are moderately toxic and those with sterilizing properties must be again considered as mutagenic and cytotoxic. Like hempa, hemel is readily demethylated by microsomal enzymes, but the resulting triazines are resistant to further attack. Such metabolites may retain some toxicity, but their mutagenic effects are reduced or entirely eliminated.

Antimetabolites.--Because the concept of antimetabolites is not used on chemical or structural similarities, no generalizations about the properties of these compounds can be made. The nonspecific antimetabolites, e.g., the folic acid antagonists, are generally cytotoxic and they must be handled with the usual precautions required for any toxic compound. The specific antimetabolites, on the other hand, may constitute an entirely different category of chemosterilants because of their predictable lack of any cytotoxic or mutagenic activity in nontarget organisms. Certain insect hormones and their analogs function as chemosterilants, but because they inhibit or interfere with processes that are unique to insects, or even to only some species of insects, they have no mutagenic or cytotoxic activity in other organisms. This specificity does not necessarily mean that such compounds would be nontoxic to other animals, but their toxicity would not involve the same systems and life processes. At this time the specific antimetabolites are primarily of research interest, and unless detailed toxicological data are available, they should be handled as potentially hazardous compounds.

STORAGE AND SHIPPING OF CHEMOSTERILANTS

As a rule, all chemosterilants should be stored in closed containers at low temperatures. The aziridines, which are the most reactive of the common chemosterilants, should never be stored in plastic or corrodible containers, and refrigeration is strongly recommended. Even at low temperatures or in solutions, the aziridines gradually decompose and materials stored for several months should be assayed before use. Any contact with acids or acidic media must be strictly avoided to prevent a possible violent decomposition. Phos= phorus amides and triazines require no special precautions and will last indefinitely when stored tightly stoppered in a cool place.

Shipping of aziridines poses numerous problems primarily owing to their low thermal stability. Unless the temperature during shipping can be controlled, the possibility of overheating and consequent decomposition must be considered. Small samples packed in vermiculite or in other insulating and absorbing material can be adequately protected, but kilogram quantities of such compounds cannot be easily handled in the same manner.

Tepa, which is one of the most reactive and thermally unstable chemo= sterilants, has been shipped as a 65- to 85-percent solution in methanol, ethanol, acetone, or methylene chloride. These solvents were claimed to retard the polymerization reaction, but their presence may create problems in using and formulating the sterilant, and their inhibitory capacity has not been clearly demonstrated. Tin-plated steel drums or glass jars were used as containers for tepa without incident for several years, but generally the shipping of large quantities of aziridines should not be attempted without expert advice. Other alkylating chemosterilants, e.g., busulfan and nonalkylating phosphorus amides or triazines, can be shipped with the usual precautions pertaining to other toxic chemicals.

HANDLING AND USE IN THE LABORATORY

As experimental compounds, chemosterilants are not difficult to handle safely. It is absolutely essential, however, to avoid ingesting them or inhaling them inadvertently. If normal safety precautions are observed, ingestion should be impossible, but the more volatile compounds, e.g., hempa, metepa, thiohempa, and to a less extent also tepa and thiotepa, could be easily inhaled. Therefore experiments in which the vapors of these compounds could reach the operator should be conducted in the fume hood or in well-ventilated rooms that need to be entered only occasionally. Table 1 shows the vapor pressures of some chemosterilants, and the data indicate the possible inhalation hazards that these compounds could produce. It should be noted, however, that in normal practice the concentration of these compounds in the atmosphere is always well below the saturation point and that only air-sampling experiments can show whether any actual danger of inhalation exposure exists.

If the skin is accidentally contaminated with a small amount of some chemosterilant, immediate and thorough washing of the area with soap and water is usually sufficient, but if the amounts are large or if blisters appear after

washing, a physician should be called immediately. Organic solvents should never be used for washing skin contaminated with any chemosterilant.

In experiments with chemosterilants, close attention must be paid to the chemical characteristics of the compounds. Alkylating agents are the most reactive of the commonly used chemosterilants, and, as mentioned earlier, any reaction leads to a decrease in their sterilizing activity. Unfortunately the potential reactivity of alkylating agents is so variable that it is seldom possible to predict whether a particular set of conditions will lead to degradation and, if so, to what extent. The effects of low pH or high temperature are the easiest to predict, but in all other instances the only reliable way to detect an interaction of the sterilant with the medium is to monitor the concentration of the compound by chemical or instrumental analysis. These considerations are particularly important in formulating alkylating chemosterilants with artificial or natural insects diets.

HANDLING AND USE IN THE FIELD

In transferring the use of chemosterilants from the laboratory to the field, two problems are usually encountered: (1) Handling large quantities of the compound and (2) preventing contamination of the environment. If chemosterilants are to be used directly against naturally occurring insects, special traps or treating stations have to be designed. Because this design may be extremely variable, each situation has to be evaluated on its own merit and no generalizations could cover all possible variations. The more probable use, however, will be the sterilization of large numbers of laboratory-reared insects and a consequent use of relatively large amounts of the chemosterilants. Most of the problems that could arise in handling large amounts of chemically or thermally unstable chemosterilants have already been discussed under storage and shipping.

In small-scale laboratory experiments the question of purity of the stock material and of its stability during formulation and administration is often neglected. This neglect is regrettable but in larger experiments it cannot be tolerated. Appropriate analytical control must be an integral part of any mass treatment and specialized literature should be consulted to determine whether chemical, physical, or biological assay methods will best serve for monitoring the entire operation. All three types of procedures are available for some alkylating agents (table 1) and the choice depends on local conditions and facilities. Except for hempa, special analytical techniques have not been elaborated for nonalkylating chemosterilants.

Whenever a chemical compound is being used, some of it will inevitably contaminate the surrounding area. Insects treated with a chemosterilant will carry the compound in their bodies and disseminate it with their excreta and regurgitated material. On the other hand, some of the compound will decompose in contact with air and water, some will be metabolized by the insect, and some will be degraded by other organisms. Consequently, the amounts of the compound in the environment of the experiment are variable and the extent of the variations must be known before the safety of the operation can be assessed. Although each situation presents a unique set of conditions that

requires individual consideration, certain generalizations concerning the chemosterilant and the method of its application are useful in estimating the potential problems in any field experiment.

Of the physical properties of the chemosterilant, solubility and vapor pressure are the most important. Compounds that are soluble in water will tend to disperse in all organisms and their residues will be washed off and diluted by rain or irrigation. Insoluble compounds, on the other hand, may accumulate and concentrate in organic materials or they may aggregate mechanically. High vapor pressure and the consequent volatility of a chemosterilant may become hazardous if insects are treated, or if treated insects are kept, in poorly ventilated areas. As table 1 shows, hempa, tepa, metepa, thiohempa, and thiotepa should be regarded as volatile and they must not be used unless the danger of inhaling air saturated with their vapors can be eliminated.

Chemical characteristics are important primarily as indicators of persistence of the sterilant and as limiting conditions imposed on its use. As discussed in the preceding section, the reactivity of alkylating agents determines how they are formulated, but with regard to residues and metabolism, each compound has to be studied individually.

Before large numbers of chemosterilized insects are released, two questions have to be answered: How much of the residual sterilant is carried by each insect at release time and how rapidly will the residue diminish during the insect's lifetime? With current analytical techniques, these questions can be answered without great difficulty and the answers should provide a rational perspective for estimating any possible hazards to people, animals, and plants.

It should be kept in mind that the quantities of residues in released insects are usually extremely small and that the potential contamination may be only a few milligrams of a compound per acre. Nevertheless predation or other natural processes could lead to unusual concentrations of undesirable compounds and the significance of such occurrences can never be evaluated in the absence of the exact data on residues and their fate.

Extensive field experiments in which kilogram quantities of a chemosterilant are to be used must not be attempted without a thorough review of the mammalian and environmental toxicology of the compound and without preparations for monitoring the fate of the chemosterilant during the experiment. The kind of toxicological data that should be collected before the experiment is started is always somewhat arbitrary. Nevertheless the following toxicity tests are essential in estimating the most critical hazards that the compound could produce:

(1) Acute oral toxicity (LD_{50}) in rats
(2) Acute dermal toxicity in rabbits
(3) Skin irritation in rabbits
(4) Eye irritation in rabbits
(5) Acute inhalation toxicity (LD_{50}) in rats
(6) Chronic toxicity in rats by repeated oral administration for 90 days

Based on the data from these tests, a rational decision can be made about the degree of safety of each operation involving the chemosterilant and also about any other toxicological tests that may be necessary.

With regard to environmental toxicology, generalizations are less satisfactory because the experimental area could be a densely populated city or a desert and the insect could be an aphid or a giant beetle. In general, when chemosterilized insects are to be released, the possibility of transferring the compound to a predator, such as birds and fish, or to the habitat of the insect, as plants and animals, should be considered. No generalizations are possible concerning other uses of chemosterilants.

The metabolism of chemosterilants presents additional problems when potential hazards of a large-scale application are to be evaluated. In released sterile insects, the metabolites of a sterilant often constitute a major component of the insect's chemical burden and thus cannot be eliminated from toxicological considerations. Fortunately the metabolic fate of most of the important chemosterilants has been studied in at least one species of insects and the analytical procedures are usually adaptable to other species. In general, the toxicological spectrum appears favorable because the mutagenic and cytotoxic properties of chemosterilants are usually destroyed or substantially reduced by metabolic processes in the insect.

The preceding considerations do not present a complete or universally applicable set of basic toxicological tests. Federal, State, or institutional regulations may prescribe different and perhaps more stringent conditions for conducting field experiments with potentially hazardous compounds. However, in the absence of such regulations, serious attention should be given to personal and environmental safety whenever larger amounts of chemosterilants are to be used. This report may serve as an initial guide.

CONCLUSION

Chemosterilants can be handled safely and effectively in the laboratory and in the field. The extreme propositions, i.e., that chemosterilants are no more harmful than aspirin, or that they are more dangerous than radioactive fallout from an atomic bomb, are equally irresponsible and wrong. All chemical compounds require respect and understanding and, as a rule, a small excess of caution is preferable to carelessness. Generalizations can never provide a complete description, and anyone intending to use chemosterilants should first study the literature in which the compounds and their properties are described in detail (4, 21).

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